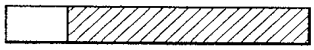
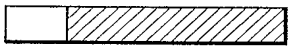
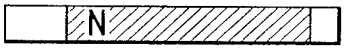
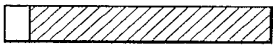
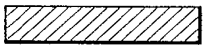
Binding Domain Hybrid		Activation Domain Hybrid
		Sentrin
Fas		
	wt (191–319AA)	++
	Δ 15 (191–304AA)	++
	Δ 23 (191–296AA)	–
	(V238N)	–
TNFR1		
	wt (326–426AA)	++
	Δ 14 (326–412AA)	++
	Δ 20 (326–406AA)	–
CD40		
	(216–277AA)	–
FADD/MORT1		
	(1–208AA)	–

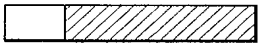
Activation Domain Hybrid		Binding Domain Hybrid
		Fas (191–319AA)
Sentrin 	(1–101AA)	++
	(1–70AA)	–
	(1–23AA)	–
	(24–97AA)	–
Ubiquitin 	(1–76AA)	–
Nedd8 	(1–76AA)	–

FIG. 1A

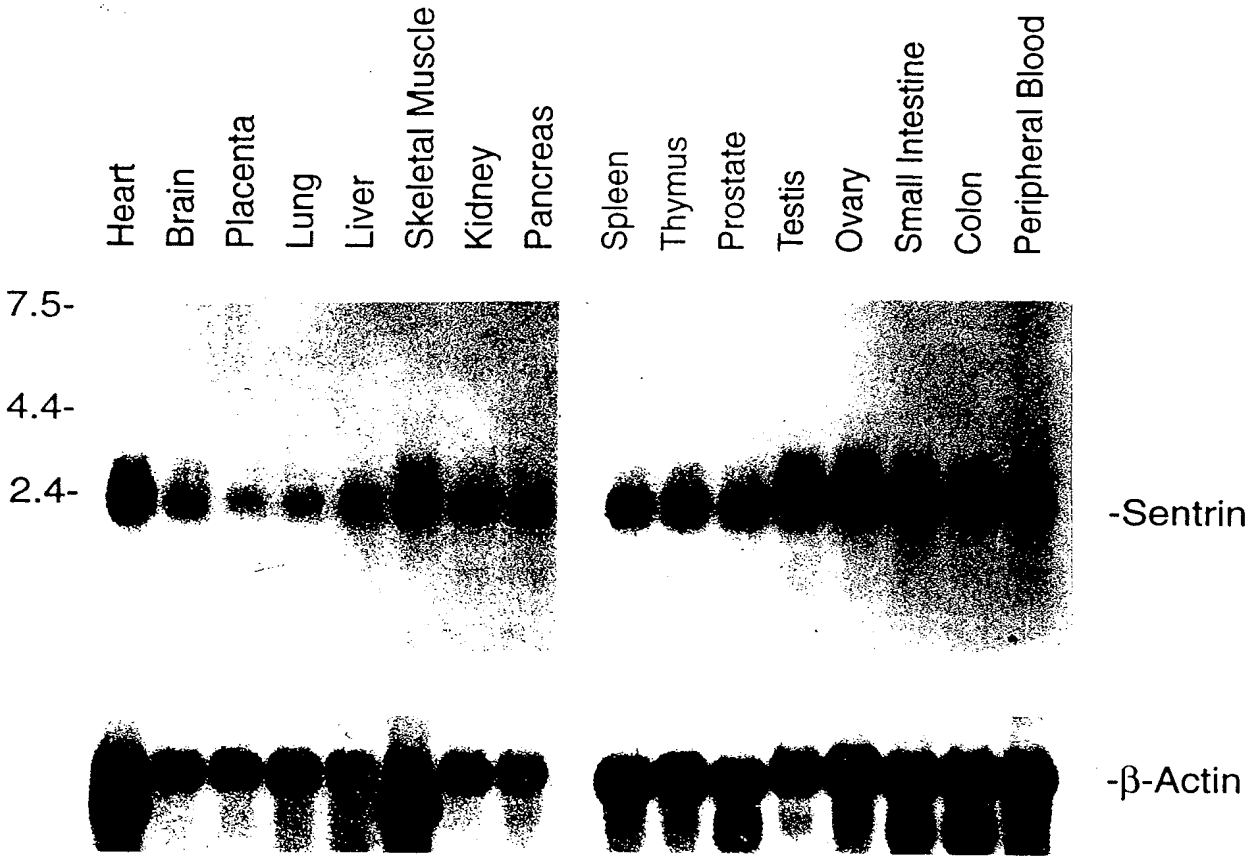

Heart
Brain
Placenta
Lung
Liver
Skeletal Muscle
Kidney
Pancreas
Spleen
Thymus
Prostate
Testis
Ovary
Small Intestine
Colon
Peripheral Blood
7.5-
4.4-
2.4-
-Sentrin
-β-Actin

FIG. 1B

CGAGGCGTAGCGGAAGTTACTGCAGCCGCGGTGTTGTGCTGT
CGGGAAGGGGAAGGATTTGTAAACCCCGGAGCGAGGTTCTGC
TTACCCGAGGCCGCTGCTGTGCGGAGACCCCCGGGTGAAGCC
ACCGTCATCATGTCTGACCAGGAGGCAAAACCTTCAACTGAG
M S D Q E A K P S T E
GACTTGGGGGATAAGAAGCAAGGTGAATATATTAAACTCAAA
D L G D K K E G E Y I K L K
GTCATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAA
V I G Q D S S E I H F K V K
ATGACAACACATCTCAAGAAACTCAAAGAATCATACTGTCAA
M T T H L K K L K E S Y C Q
AGACAGGGTGTTCCAATGAATTCACTCAGGTTTCTCTTTGAG
R Q G V P M N S L R F L F E
GGTCAGAGAATTGCTGATAATCATACTCCAAAAGAACTGGGA
G Q R I A D N H T P L E L G
ATGGAGGAAGAAGATGTGATTGAAGTTTATCAGGAACAAACG
M E E E D V I E V Y Q E Q T
GGGGGTCATTCAACAGTTTAGATATTCTTTTTATTTTTTTTC
G G H S T V *101
TTTTCCCTCAATCCTTTTTTATTTTTAAAAATAGTTCTTTTC
TAATGTGGTGTTCAAAACGGAATTGAAAACTGGCACCCCATC
TCTTTGAAACATCTGGTAATTTGAATTCTAGTGCTCATTATT
CATTATTGTTTGTTTTCATTGTGCTGATTTTTGGTGATCAAG
CCTCAGTCCCCTTCATATTACCCTCTCCTTTTTAAAAATTAC
GTGTGCACAGAGAGGTCACCTTTTTCAGGACATTGCATTTTC
AGGCTTGTGGTGATAAATAAGATCGACCAATGCAAGTGTTCA
TAATGACTTTCCAATTGGCCCTGATGTTCTAGCATGTGATTA
CTTCACTCCTGGACTGTGACTTTCAGTGGGAGATGGAAGTTT
TTCAGAGAACTGAACTGTGGAAAAATGACCTTTCCTTAACTT
GAAGCTACTTTTAAAATTGAGAGTAATGACTAACTCCAAAGA
TGGCTTCACTGAAGAAAAGGCATTTTAAGATTTTTTAAAAAT
CTTGTCAGAAGATCCCAGAAAAGTTCTAATTTTCATTAGCAA

FIG. 2A-1

TTAATAAAGCTATACATGCAGAAATGAATACAACAGAACACT
GCTCTTTTTGATTTTATTTGTACTTTTTGGCCTGGGATATGG
GTTTTAAATGGACATTGTCTGTACCAGCTTCATTAAAATAAA
CAATATTTGTCAAAAATCGTACTAATGCTTATTTTATTTTAA
TTGTATAGAAAGAAAAAAATGCCTAAAATAAGGTTTTCTTGC
ATAAATACTGGAAATTGCACATGGTACAAAAAAAAAATGCCT
AAATTACTGTACAGGGATGATGTTAATGACTTTGGAGCACTG
AAAGTTACTGAAGTGCCTTCTGAATCAAGGATTTAATTAAGG
CCACAATACCTTTTTAATACTCAGTGTTCTGTTTTTTTTAAA
AACTTGATATTCCCGTATGGTGCATATTTGATACAGGTACCC
AATCATGTTGGATAAATGGGCATGCCAGCC

FIG. 2A-2

```
                1                                                   40
Sentrin         MSD    QEAKPST EDLGDKKEGE YIKLKVIGQD SSEIHFKVKM
SMT3            MSDSEVNQEAKPEV KP-EVKPETH -INLKV-SDG SSEIFFKIKK
Ubiquitin                                  MQIFVKTLT GKTITLEVEP
Nedd-8                                     MLIKVKTLT GKEIEIDIEP

                                           41                  60
Sentrin                                    TTHLKKLKES YCQRQGVPMN
SMT3                                       TTPLRRLMEA FAKRQGKEMD
Ubiquitin                                  SDTIENVKAK IQDKEGIPPD
Nedd-8                                     TDKVERIKER VEEKEGIPPQ
BAG-1                                      ---VQDLAQL VEEATGVPLP

                                           61                  80
Sentrin                                    SLRFLFEGQR IADNHTPKEL
SMT3                                       SLRFLYDGIT IQADQTPEDL
Ubiquitin                                  QQRLIFAGKQ LEDGRTLSDY
Nedd-8                                     QQRLIYSGKQ MNDEKTAADY
BAG-1                                      FQKLIFKGKS LKE-------

                                           81                 100
Sentrin                                    GMEEEDVIEV YQEQTGGHST V
SMT3                                       DMEDNDIIEA HREQIGGATY
Ubiquitin                                  NIQKESTLHL VLRLRGG
Nedd-8                                     KILGGSVLHL VLALRGG
```

FIG. 2B

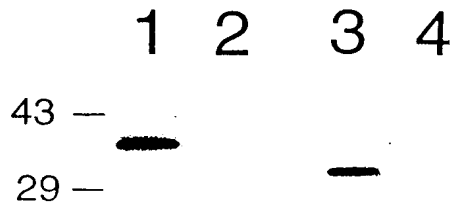


FIG.3

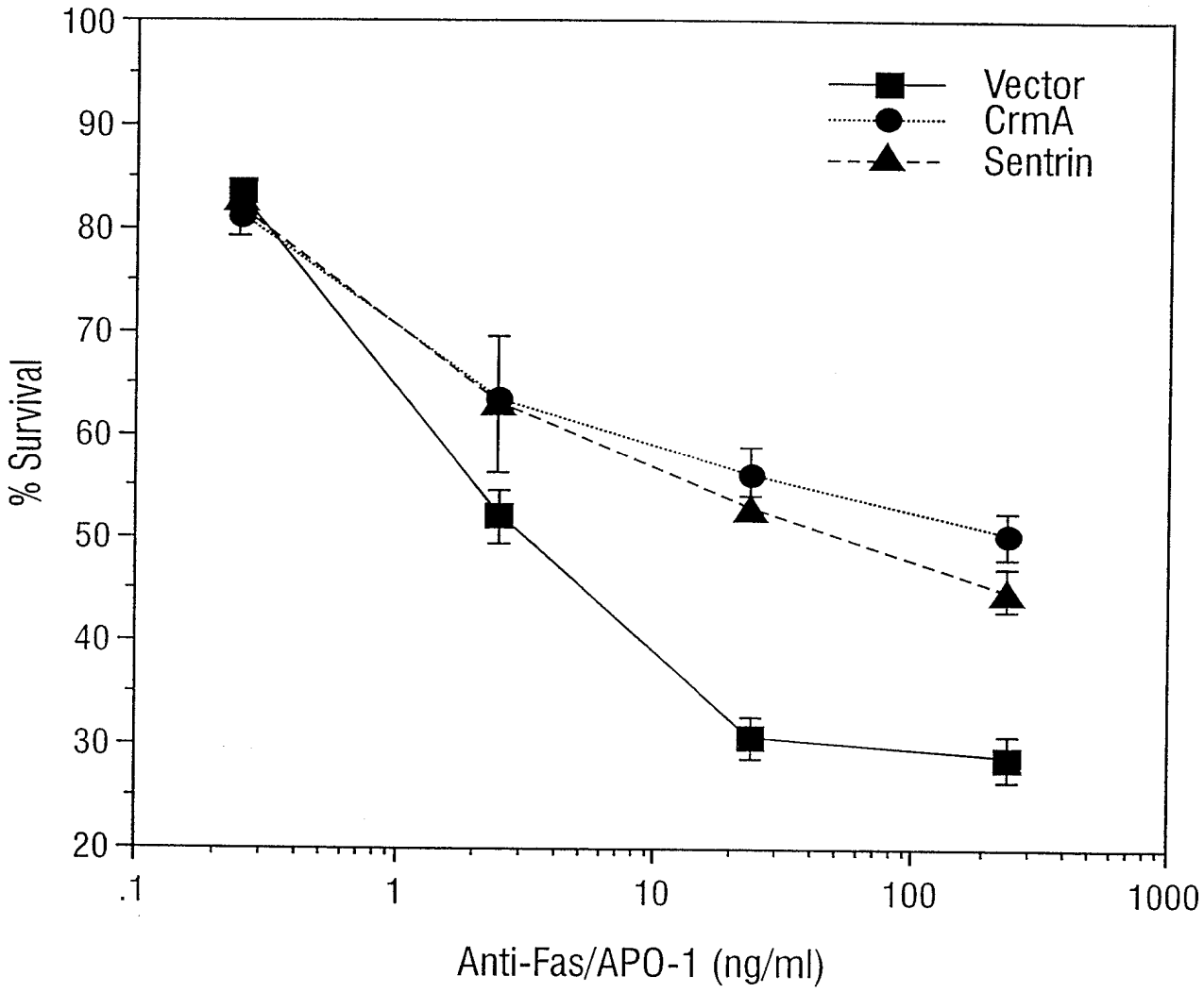

100
90
80
70
60
50
40
30
20
% Survival
.1
1
10
100
1000
Anti-Fas/APO-1 (ng/ml)
Vector
CrmA
Sentrin

FIG. 4A

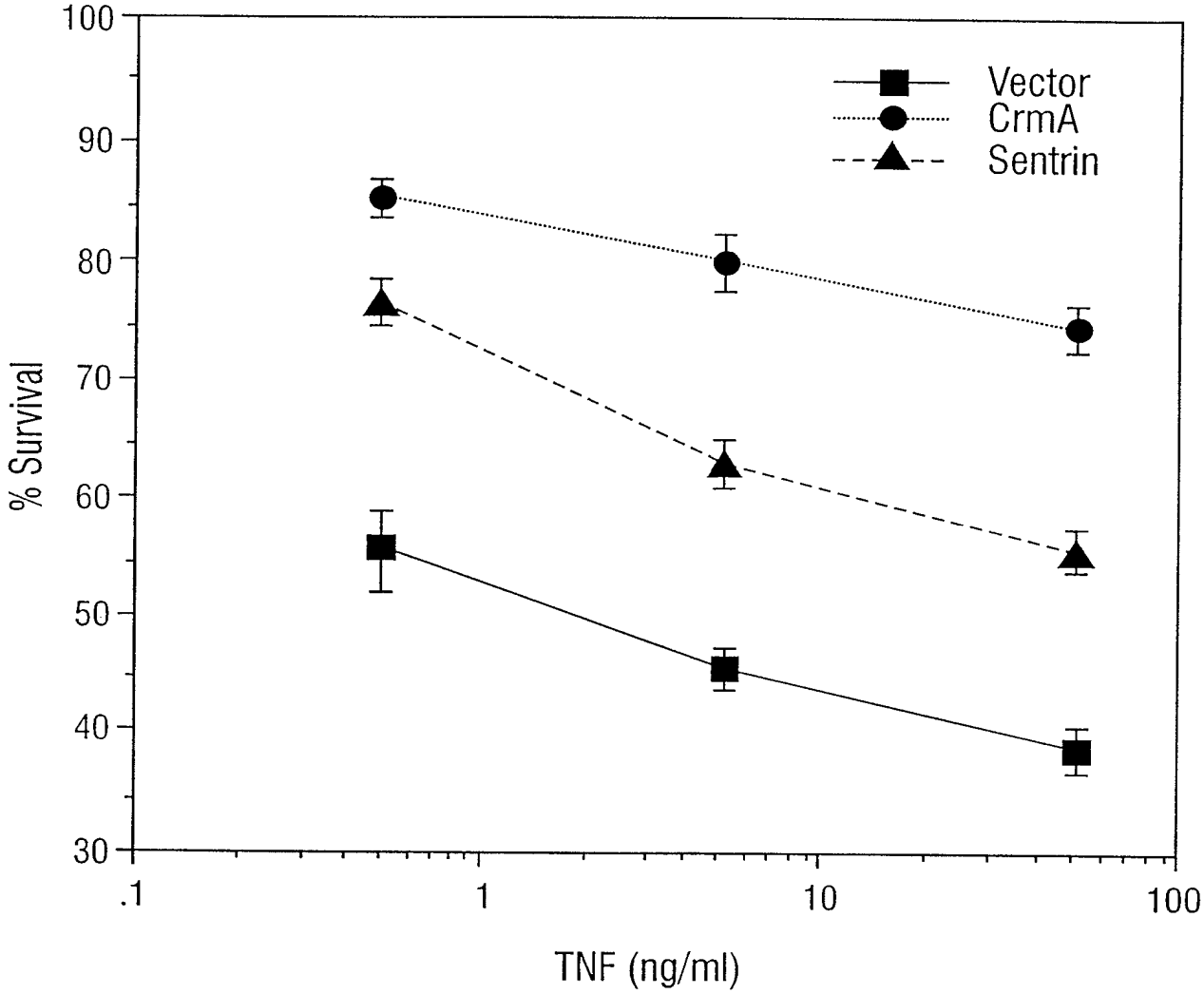

100
90
80
70
60
50
40
30
% Survival
.1
1
10
100
TNF (ng/ml)
Vector
CrmA
Sentrin

FIG. 4B

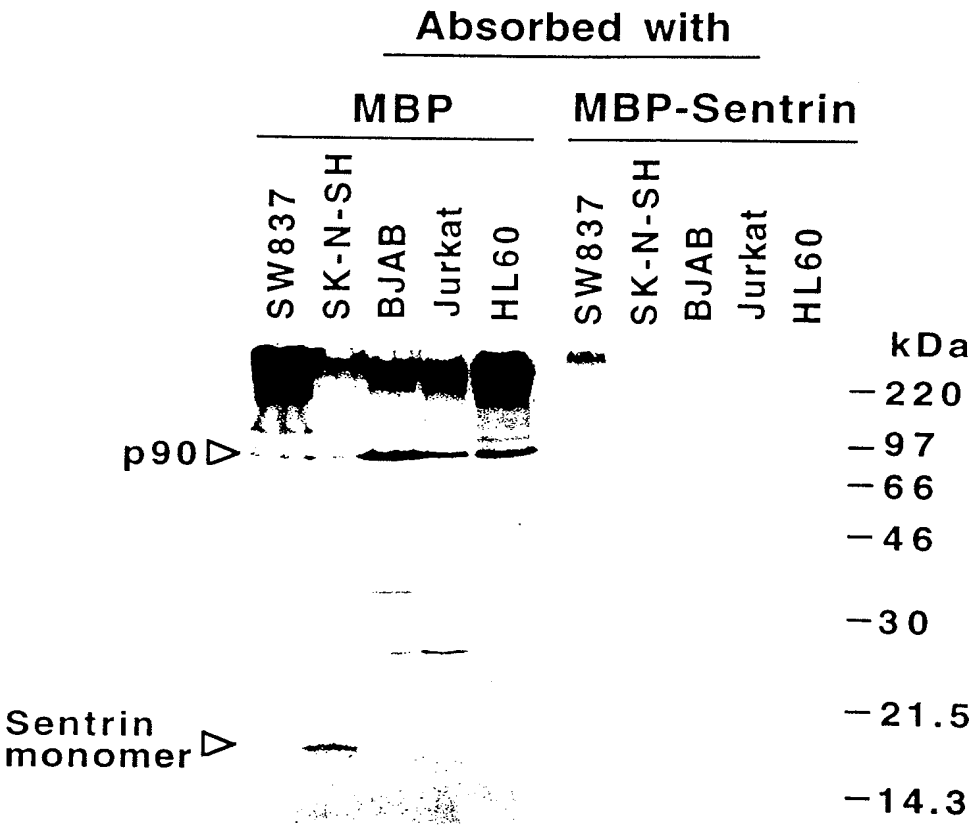

Absorbed with
MBP
MBP-Sentrin
SW837
SK-N-SH
BJAB
Jurkat
HL60
SW837
SK-N-SH
BJAB
Jurkat
HL60
kDa
220
97
66
46
30
21.5
14.3
p90
Sentrin monomer

FIG.5

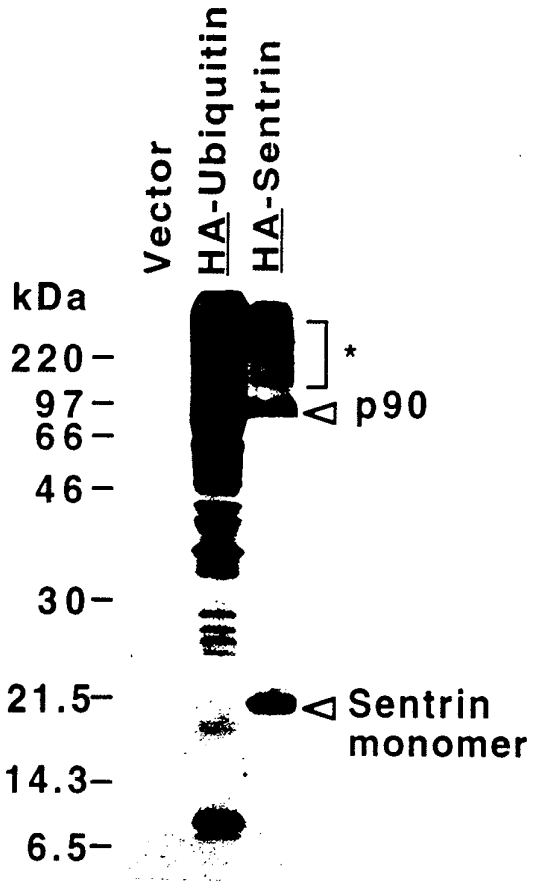

Vector
HA-Ubiquitin
HA-Sentrin
kDa
220
97
66
46
30
21.5
14.3
6.5
*
p90
Sentrin
monomer

FIG.6A

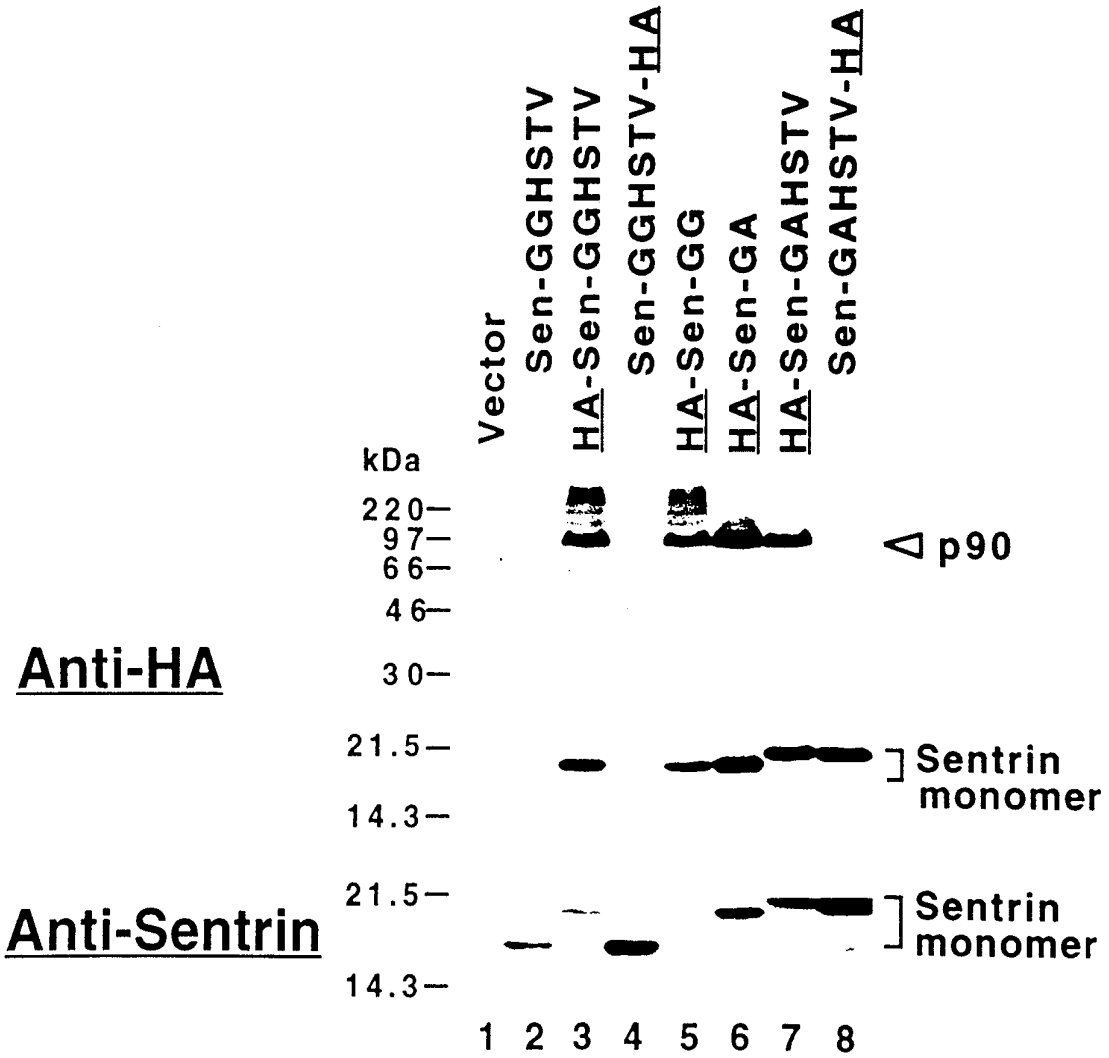

Vector
Sen-GGHSTV
HA-Sen-GGHSTV
Sen-GGHSTV-HA
HA-Sen-GG
HA-Sen-GA
HA-Sen-GAHSTV
Sen-GAHSTV-HA
kDa
220
97
66
46
30
21.5
14.3
p90
Anti-HA
Sentrin
monomer
Anti-Sentrin
Sentrin
monomer
1 2 3 4 5 6 7 8

FIG.6B

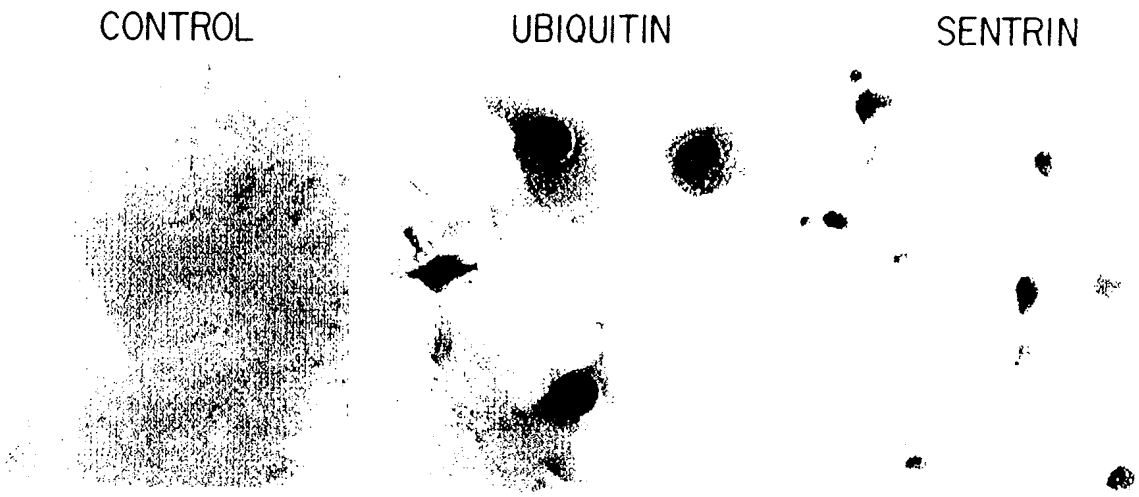

CONTROL
UBIQUITIN
SENTRIN

FIG. 7A

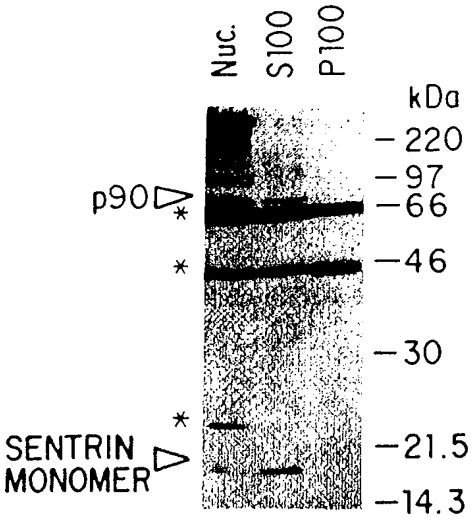

Nuc.
S100
P100
kDa
220
97
66
46
30
21.5
14.3
p90
SENTRIN
MONOMER

FIG. 7B

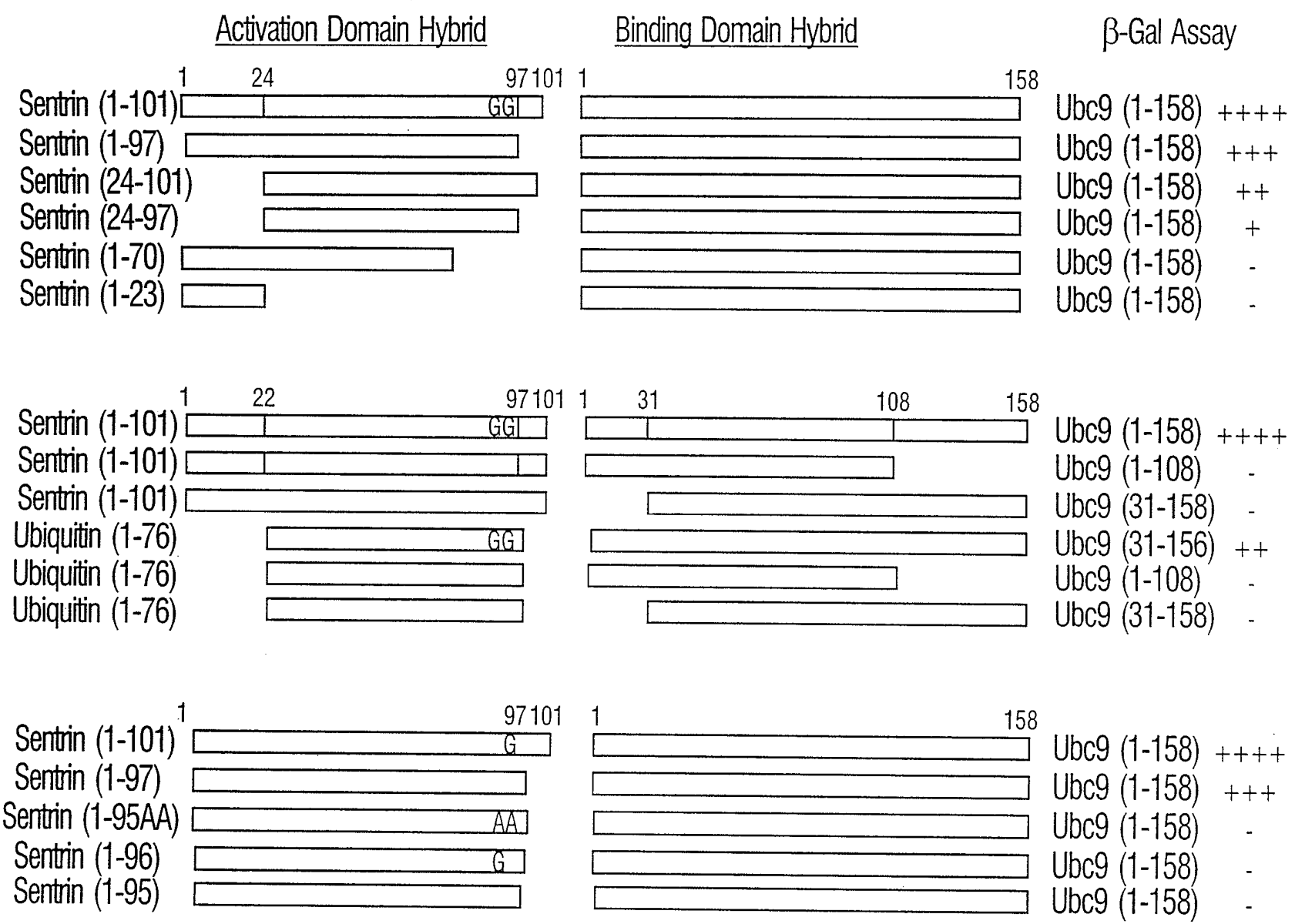

Activation Domain Hybrid
Binding Domain Hybrid
β-Gal Assay
1
24
97 101
GG
Sentrin (1-101)
Sentrin (1-97)
Sentrin (24-101)
Sentrin (24-97)
Sentrin (1-70)
Sentrin (1-23)
1
158
Ubc9 (1-158) ++++
Ubc9 (1-158) +++
Ubc9 (1-158) ++
Ubc9 (1-158) +
Ubc9 (1-158) -
Ubc9 (1-158) -
1
22
97 101
GG
Sentrin (1-101)
Sentrin (1-101)
Sentrin (1-101)
Ubiquitin (1-76)
Ubiquitin (1-76)
Ubiquitin (1-76)
1
31
108
158
Ubc9 (1-158) ++++
Ubc9 (1-108) -
Ubc9 (31-158) -
Ubc9 (31-156) ++
Ubc9 (1-108) -
Ubc9 (31-158) -
1
97 101
G
AA
G
Sentrin (1-101)
Sentrin (1-97)
Sentrin (1-95AA)
Sentrin (1-96)
Sentrin (1-95)
1
158
Ubc9 (1-158) ++++
Ubc9 (1-158) +++
Ubc9 (1-158) -
Ubc9 (1-158) -
Ubc9 (1-158) -

FIG. 8

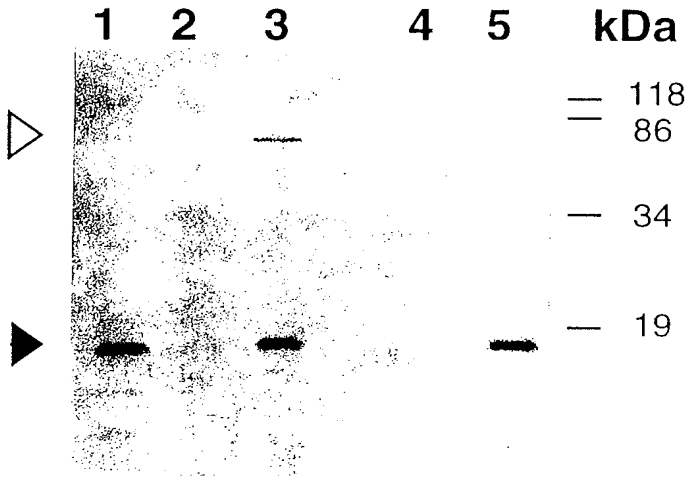


FIG.9

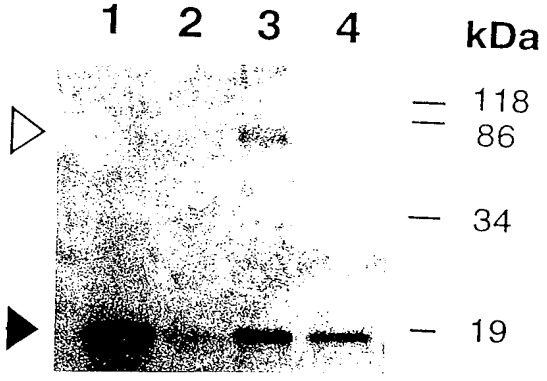


FIG.10

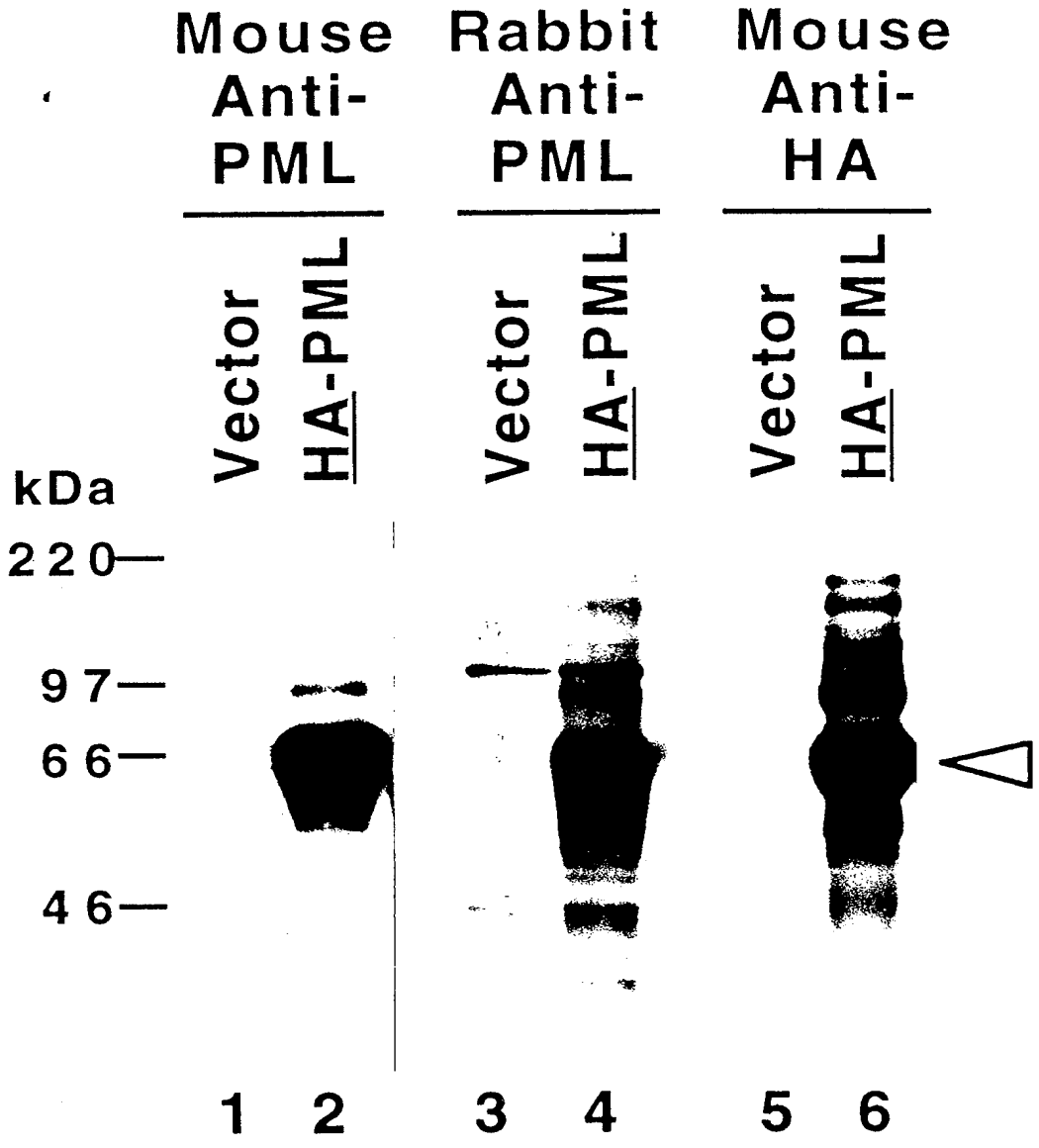

Mouse Anti-PML
Rabbit Anti-PML
Mouse Anti-HA
Vector
HA-PML
Vector
HA-PML
Vector
HA-PML
kDa
220
97
66
46
1 2 3 4 5 6

FIG.11A

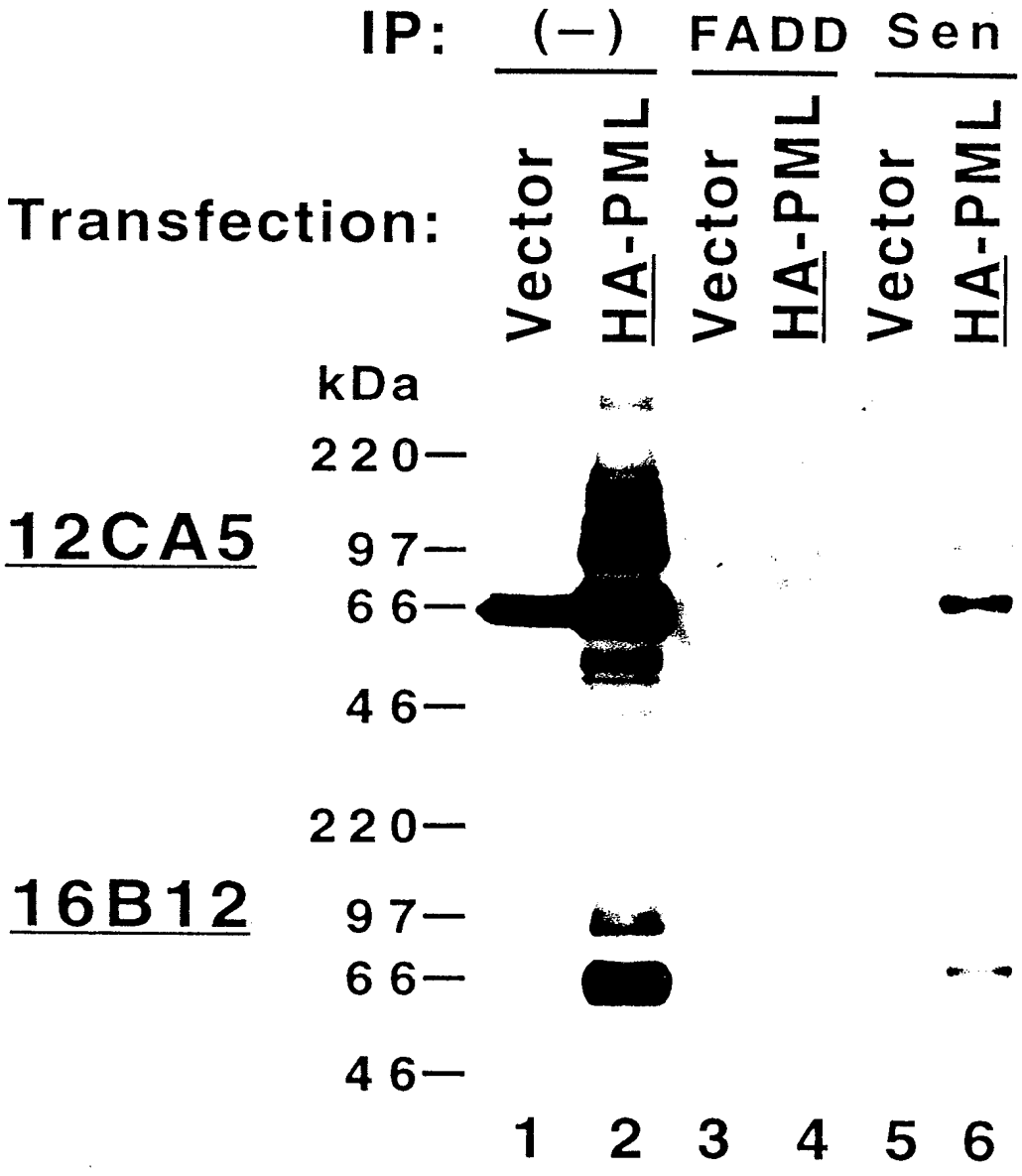

IP:
(−)
FADD
Sen
Transfection:
Vector
HA-PML
Vector
HA-PML
Vector
HA-PML
kDa
220—
97—
66—
46—
12CA5
220—
97—
66—
46—
16B12
1 2 3 4 5 6

FIG.11B

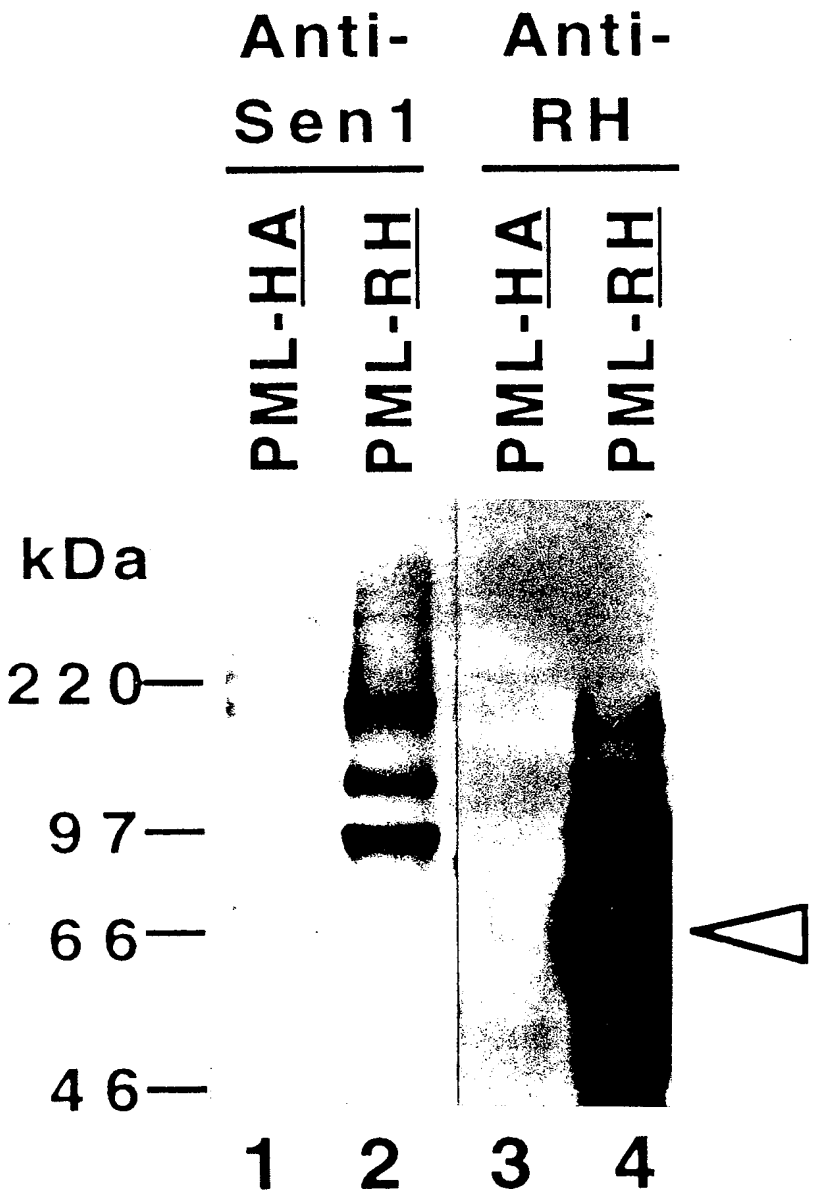

Anti-Sen1
Anti-RH
PML-HA
PML-RH
PML-HA
PML-RH
kDa
220
97
66
46
1
2
3
4

FIG.11C

Sentrin-1(1-30)	MSDQEAKPST	EDLGDKKEGE	-YIKLKVIGQD
Sentrin-2(1-26)	MAD-E-KPK-	E--GVKTENN	DHINLKVAGQD
Sentrin-3(1-25)	MSE-E-KPK-	E--GVKTEN-	DHINLKVAGQD
NEDD8(1-9)			MLIKVKTLT
Ubiquitin(1-9)			MQIFVKTLT

Sentrin-1(31-60)	SSEIHFKVKM	TTHLKKLKES	YCQRQGVPMN
Sentrin-2(27-56)	GSVVQFKIKR	HTPLSKLMKA	YCERQGLSMR
Sentrin-3(26-55)	GSVVQFKIKR	HTSLSKLMKA	YCERQGLSMR
NEDD8(10-39)	GKEIEIDIEP	TDKVERIKER	VEEKEGIPPQ
Ubiquitin(10-39)	GKTITLEVEP	SDTIENVKAK	IQDKEGIPPD

Sentrin-1(61-90)	SLRFLFEGQR	IADNHTPKEL	GMEEEDVIEV
Sentrin-2(57-86)	QIRFRFDGQP	INETDTPAQL	EMEDEDTIDV
Sentrin-3(56-85)	QIRFRFDGQP	INETDTPAQL	RMEDEDTIDV
NEDD8(40-69)	QQRLIYSGKQ	MNDEKTAADY	KILGGSVLHL
Ubiquitin(40-69)	QQRLIFAGKQ	LEDGRTLSDY	NIQKESTLHL

Sentrin-1(91-101)	YQEQTGGHSTV
Sentrin-2(87-95)	FQQQTGGVY
Sentrin-3(86-103)	FQQQTGGVPESSLAGHSF
NEDD8(70-81)	VLALRGGGGLR
Ubiquitin(70-76)	VLRLRGG

FIG. 12

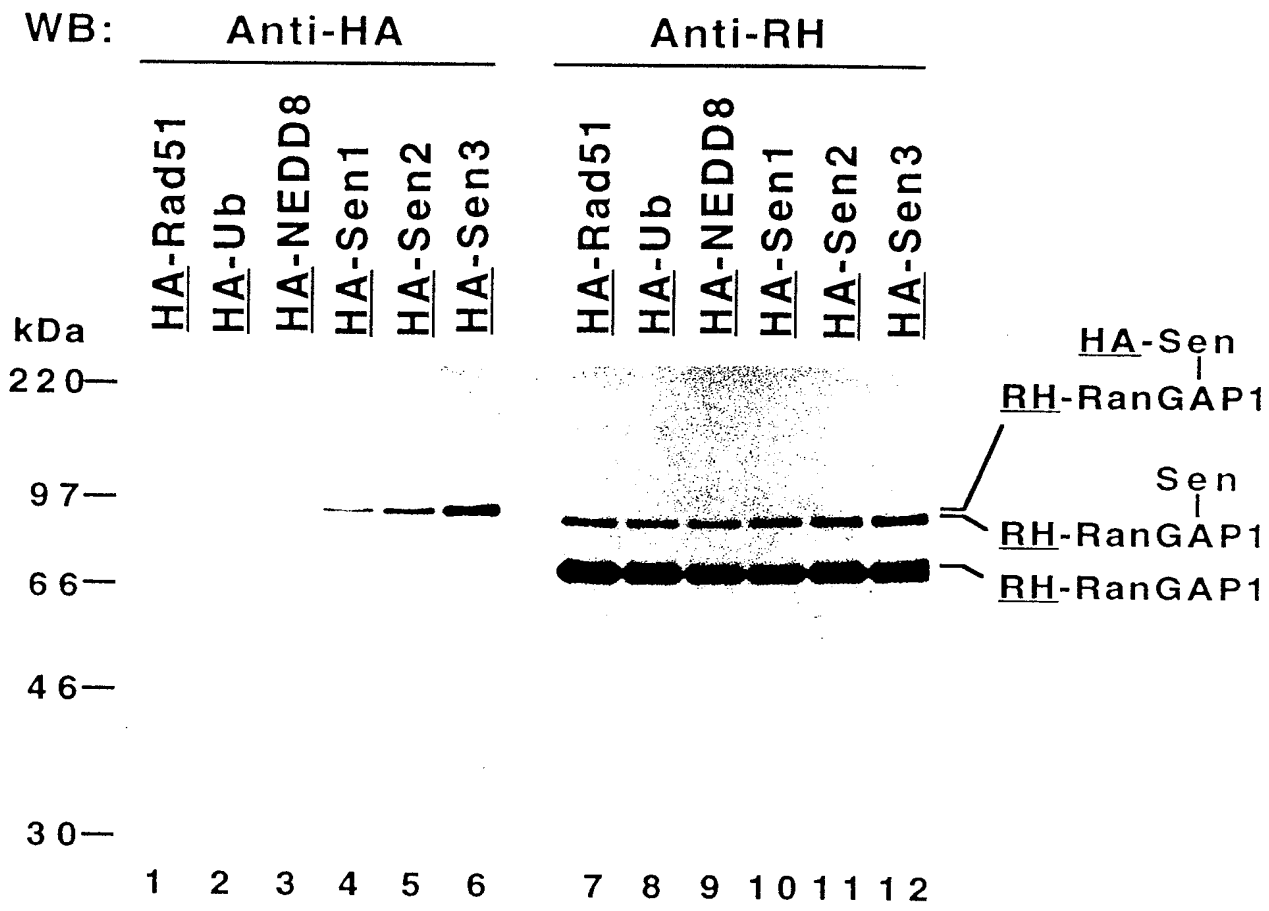

WB:
Anti-HA
Anti-RH
HA-Rad51
HA-Ub
HA-NEDD8
HA-Sen1
HA-Sen2
HA-Sen3
HA-Rad51
HA-Ub
HA-NEDD8
HA-Sen1
HA-Sen2
HA-Sen3
kDa
220—
97—
66—
46—
30—
HA-Sen
RH-RanGAP1
Sen
RH-RanGAP1
RH-RanGAP1
1 2 3 4 5 6 7 8 9 10 11 12

FIG.13A

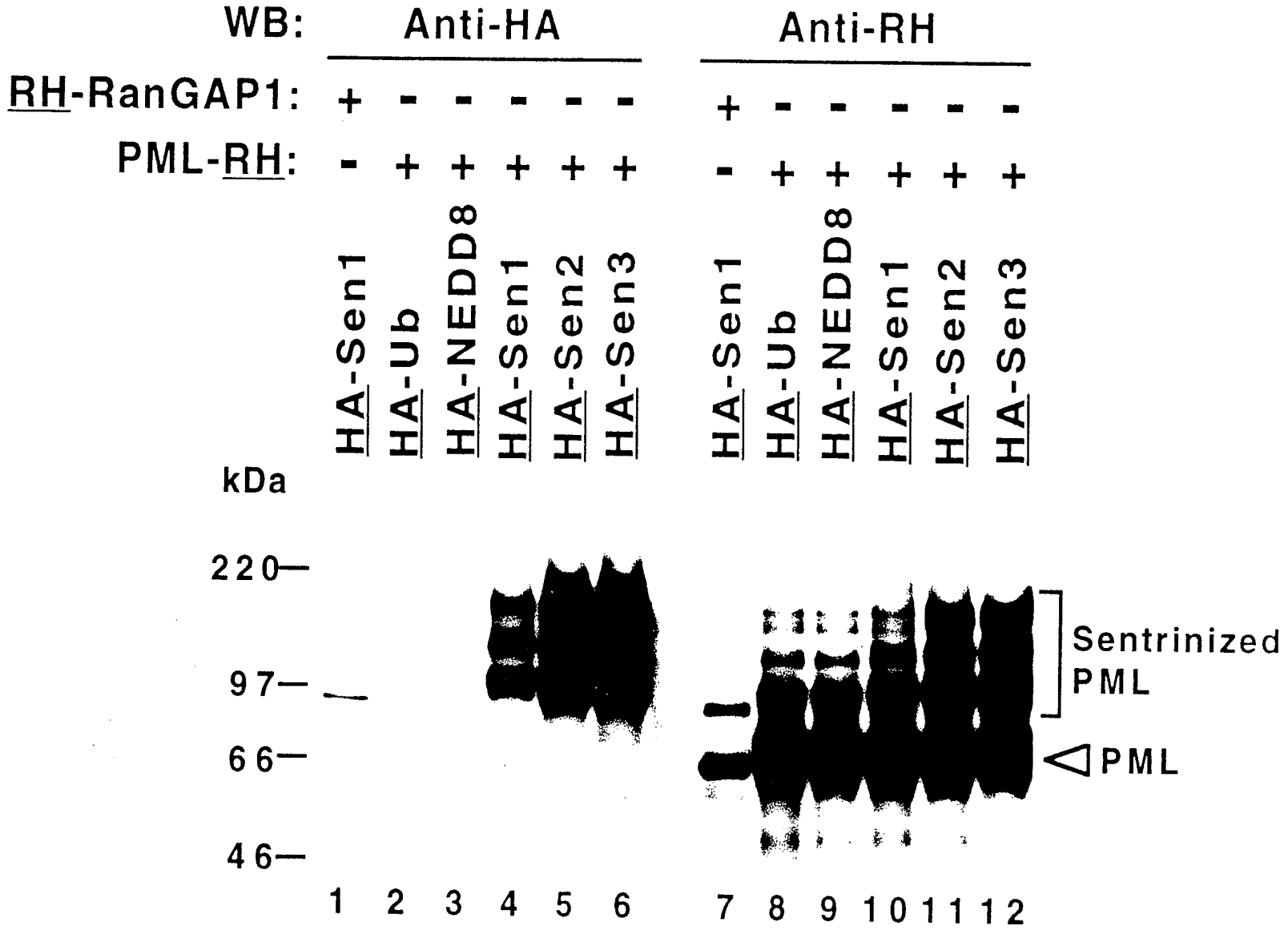

WB:
Anti-HA
Anti-RH
RH-RanGAP1:
PML-RH:
HA-Sen1
HA-Ub
HA-NEDD8
HA-Sen1
HA-Sen2
HA-Sen3
HA-Sen1
HA-Ub
HA-NEDD8
HA-Sen1
HA-Sen2
HA-Sen3
kDa
220
97
66
46
Sentrinized PML
PML
1 2 3 4 5 6 7 8 9 10 11 12

FIG.13B

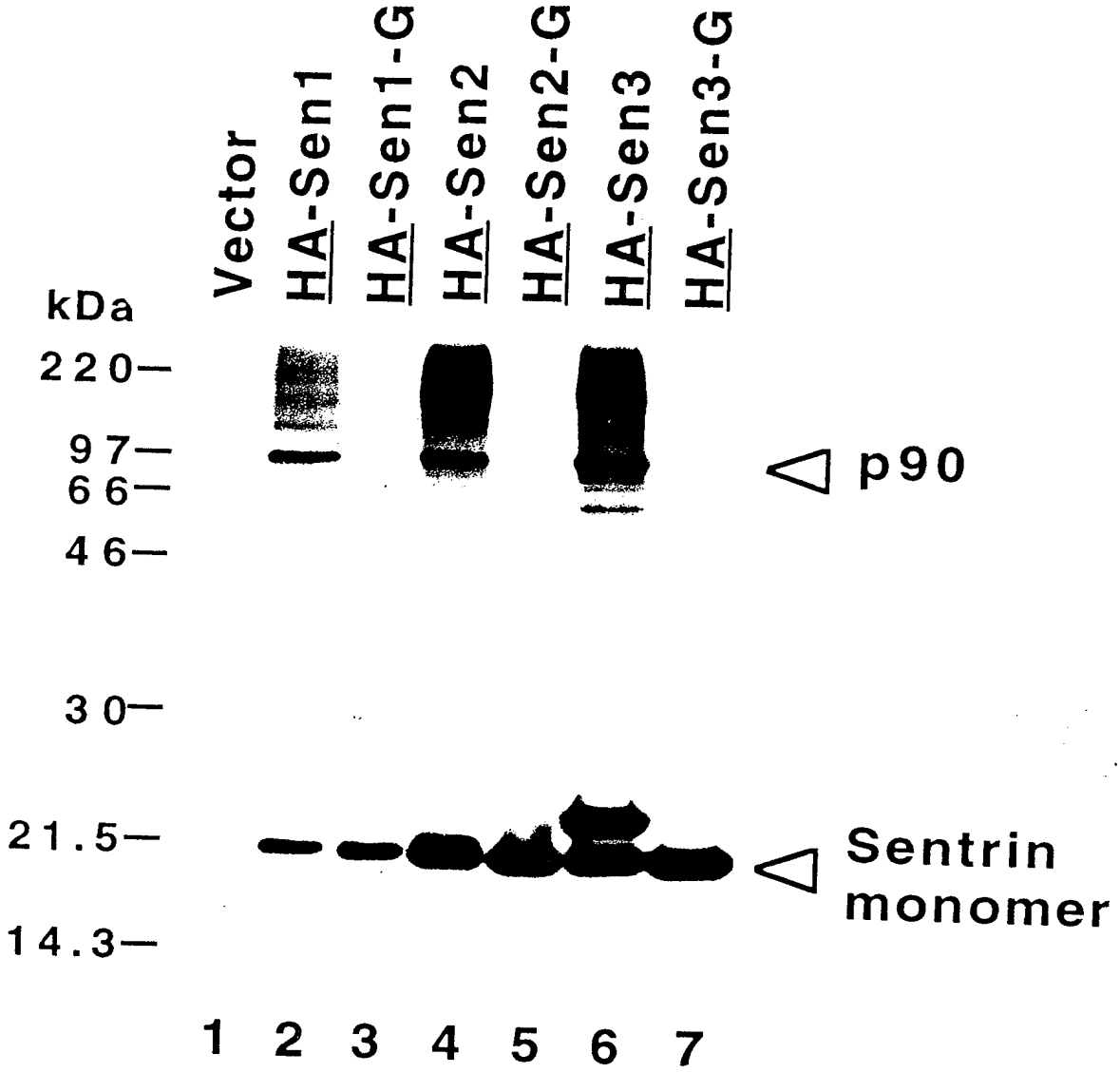

Vector
HA-Sen1
HA-Sen1-G
HA-Sen2
HA-Sen2-G
HA-Sen3
HA-Sen3-G
kDa
220
97
66
46
30
21.5
14.3
p90
Sentrin monomer
1 2 3 4 5 6 7

FIG.13C

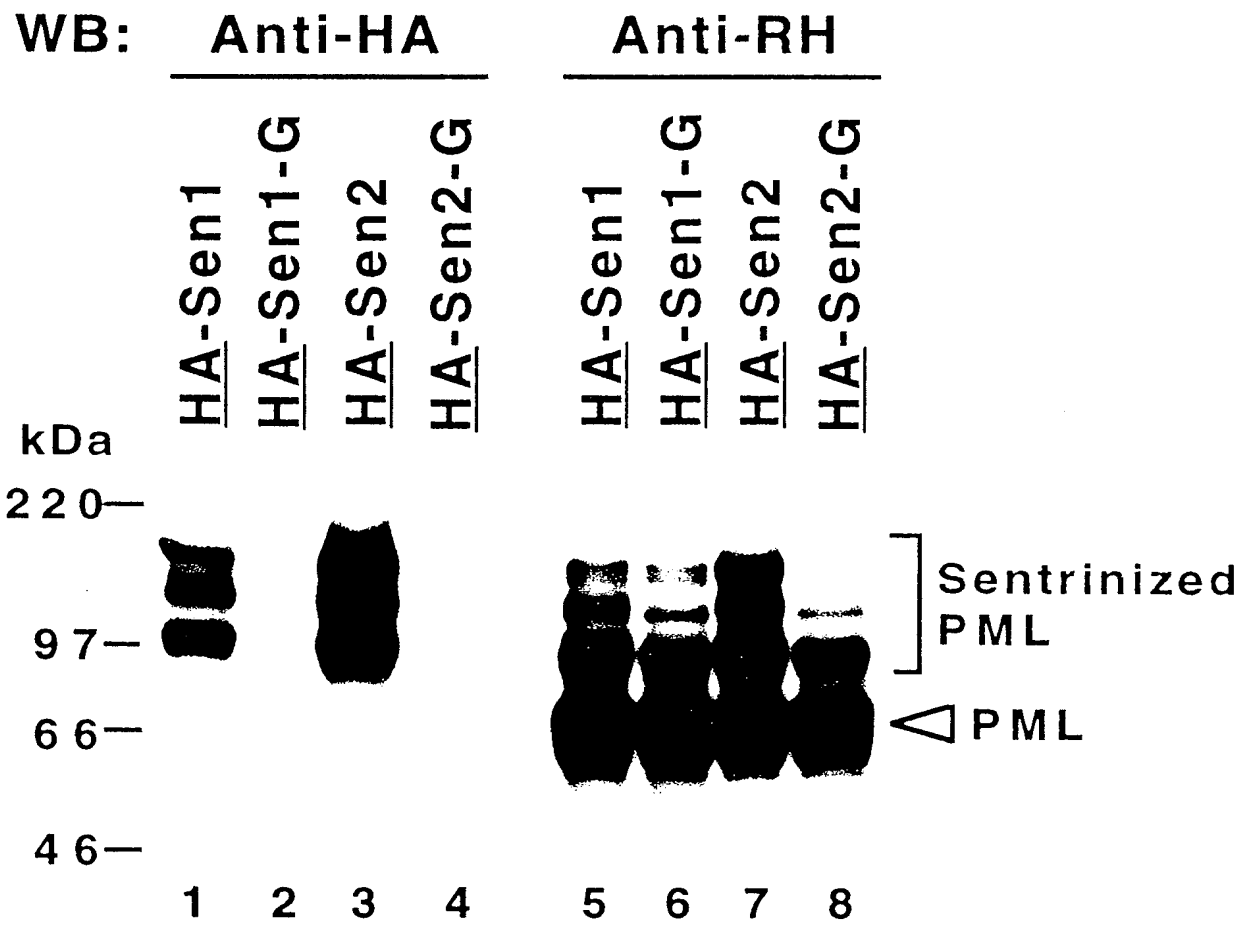

WB:
Anti-HA
Anti-RH
HA-Sen1
HA-Sen1-G
HA-Sen2
HA-Sen2-G
HA-Sen1
HA-Sen1-G
HA-Sen2
HA-Sen2-G
kDa
220
97
66
46
Sentrinized PML
PML
1
2
3
4
5
6
7
8

FIG.13D